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Evaluation of Two Immunoblot Assays and a Western Blot Assay for the Detection of Antisyphilis Immunoglobulin G Antibodies[▽]

Ryan J. Welch[1]* and Christine M. Litwin[1,2]

Associated Regional and University Pathologists (ARUP) Institute for Clinical and Experimental Pathology, Salt Lake City, Utah,[1] and Department of Pathology, University of Utah, Salt Lake City, Utah[2]

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In the present study, two immunoglobulin G (IgG) immunoblot assays and one IgG Western blot assay were compared to the rapid plasma reagin test (RPR), the fluorescent treponemal antibody absorption test (FTA-ABS), and the *Treponema pallidum* particle agglutination assay (TP-PA). The agreement levels of the Viramed, Virotech, and MarDx assays were 97.0%, 96.4%, and 99.4%, and the agreements of samples inconclusive by FTA-ABS and resolved by TP-PA were 91.7%, 83.3%, and 69.4%, respectively.

Syphilis, a disease caused by *Treponema pallidum*, is transmitted congenitally or through sexual intercourse (8–9). Non-treponema-based tests such as the rapid plasma reagin test (RPR) are used to detect syphilis infection (6, 9–10). These tests may produce false-positive results in pregnant women and patients with infections (3, 5–6, 9, 11). An algorithm has been developed for the serological diagnosis of syphilis which includes a non-treponema-based screening test and a treponema-based confirmatory assay (1–2, 7, 11). Traditional confirmatory assays include the fluorescent treponemal antibody absorption test (FTA-ABS) and the *T. pallidum* particle agglutination assay (TP-PA) (9).

Western blot-based assays to detect immunoglobulin G (IgG) antibodies may prove useful, especially in cases where the FTA-ABS is inconclusive. In the present study, results of two immunoblot assays and one Western blot assay were compared to FTA-ABS/TP-PA and RPR results, as well as to each other.

Human sera. A total of 200 human serum samples sent to Associated Regional and University Pathologists (ARUP) laboratories for syphilis testing were collected. Procedures were followed in accordance with the ethical standards established by the University of Utah in accordance with the Helsinki Declaration of 1975. All patient samples were deidentified according to the University of Utah Institutional Review Board protocol (no. 7275) to meet the Health Information Portability and Accountability Act guidelines. Specimens were stored at −20°C until testing and then stored at 2 to 8°C.

Non-treponema-based testing. All 200 samples were tested by RPR according to the manufacturer's protocol (Arlington Scientific, Inc., Springville, UT).

Treponema-based testing. One hundred forty-two samples were tested by FTA-ABS (Inverness Medical, Waltham, MA), and 32 inconclusive samples were further tested by TP-PA (Fujirebio, Malvern, PA). Both assays were performed according to the manufacturers' protocols. The 32 inconclusive FTA-ABS samples were included to reflect the high percentage of inconclusive FTA-ABS samples sent to our reference laboratory from primary screening laboratories.

Syphilis blot testing. All 200 samples were tested using two immunoblot assays and one Western blot assay, the Treponema ViraBlot test kit IgG (Viralab Inc., Oceanside, CA), the *Treponema pallidum* IgG line immunoblot (Genzyme Virotech GmbH, Rüsselsheim, Germany), and the *T. pallidum* IgG Marblot strip test system (MarDx Diagnostics, Inc., Carlsbad, CA). Each assay was performed according to the manufacturer's protocol.

Statistical analysis. To determine overall agreement, sensitivity, specificity, and 95% confidence intervals (CI), two-by-two contingency table analysis with Yates-corrected chi-square testing was used (4). Equivocal results were excluded from the calculations. Samples that disagreed were repeated on each test. Receiver operating characteristic (ROC) curves were analyzed using MedCalc version 10.1.3.0 (MedCalc Software, Mariakerke, Belgium).

Of the 200 samples used in this study, 142 were tested by treponema-based assays and RPR and 58 were tested exclusively by RPR. Samples were considered positive if they tested positive in the FTA-ABS assay or the TP-PA assay. Samples that were inconclusive according to the FTA-ABS assay were resolved by the TP-PA assay.

For the ViraBlot assay, the overall agreement, sensitivity, and specificity were 97.0%, 95.5% (95% CI, 90.4 to 97.9) and 97.8% (95% CI, 95.2 to 99.0%), respectively (Table 1), with no equivocal results. The Virotech assay had overall agreement, sensitivity, and specificity values of 96.4%, 90.0% (95% CI, 84.7 to 91.4%), and 99.2% (95% CI, 96.8 to 99.9%), respectively, with five (2.5%) equivocal results. The MarDx assay had overall agreement, sensitivity, and specificity values of 99.4%, 98.2% (95% CI, 94.3 to 98.2%), and 100.0% (95% CI, 98.2 to 100.0%), respectively, with 25 (12.5%) equivocal results.

To determine if the manufacturers' cutoff criteria were optimal, ROC curves were generated. The ViraBlot assay produced an ROC curve with an area under the curve (AUC) of 0.988 ($P < 0.0001$). The optimal cutoff criterion for maximum sensitivity and specificity matched the manufacturer's protocol. For the Virotech assay, an ROC curve with an AUC of 0.987 ($P < 0.0001$) was produced. This ROC curve indicated that by

* Corresponding author. Mailing address: ARUP Institute for Clinical and Experimental Pathology, 500 Chipeta Way, Salt Lake City, UT 84108. Phone: (801) 583-2787, ext. 3540. Fax: (801) 584-5048. E-mail: ryan.welch@aruplab.com.

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TABLE 1. Summary of results comparing each immunoblot and Western blot assay with the FTA-ABS/TP-PA and RPR results

Test and result	No. of samples		
	FTA-ABS/TP-PA[a]		RPR[b] negative
	Positive	Negative	
ViraBlot assay[c]			
Positive	63	2	1
Negative	3	74	57
Virotech immunoblot[d]			
Positive	57	1	0
Negative	6	73	58
Equivocal	3	2	0
Marblot strip assay[e]			
Positive	55	0	0
Negative	1	67	52
Equivocal	10	9	6

[a] FTA-ABS and TP-PA resolved results.
[b] Samples originally subjected to the RPR only.
[c] The Treponema ViraBlot test kit IgG had overall agreement, sensitivity, and specificity values of 97.0%, 95.5% (95% CI, 90.4 to 97.9), and 97.8% (95% CI, 95.2 to 99.0%), respectively.
[d] The *T. pallidum* IgG line immunoblot had overall agreement, sensitivity, and specificity values of 96.4%, 90.0% (95% CI, 84.7 to 91.4%), and 99.2% (95% CI, 96.8 to 99.9%), respectively.
[e] The *T. pallidum* IgG Marblot strip test system had overall agreement, sensitivity, and specificity values of 99.4%, 98.2% (95% CI, 94.3 to 98.2%), and 100.0% (95% CI, 98.2 to 100.0%), respectively.

TABLE 2. Summary of the 32 samples inconclusive by FTA-ABS and resolved by TP-PA

Test and result	No. of samples determined by TP-PA[a] to be:	
	Positive	Negative
ViraBlot assay[b]		
Positive	15	2
Negative	1	14
Virotech immunoblot[c]		
Positive	11	0
Negative	4	15
Equivocal	1	1
Marblot strip assay[d]		
Positive	9	0
Negative	0	12
Equivocal	7	4

[a] TP-PA resolved results.
[b] The Treponema ViraBlot test kit IgG had an accuracy of 90.6% compared with the TP-PA resolved results.
[c] The *Treponema pallidum* IgG line immunoblot had an accuracy of 81.3% compared with the TP-PA resolved results.
[d] The *T. pallidum* IgG Marblot strip test system had an accuracy of 65.6% compared with the TP-PA resolved results.

reducing the cutoff criterion by one band, the sensitivity could be increased from 90.0% to 98.4% (95% CI, 93.6 to 99.7%) without significantly decreasing the specificity. This would reduce the number of false-negative results. The Marblot assay produced an ROC curve with an AUC of 0.988 ($P < 0.0001$). The ROC curve indicated that by reducing the cutoff criterion by one, the number of equivocal results would decrease from 25 to 14 without significantly decreasing sensitivity or specificity. However, this is still an unacceptably high number of equivocal samples.

Although FTA-ABS testing offers high sensitivity and specificity, the subjectivity of the test results in a high number of inconclusive results (9). From June 2008 to June 2009 at ARUP laboratories, 7.8% of the samples run by FTA-ABS were reported as inconclusive and subsequently assayed by TP-PA, a major extra cost. Therefore, the development goal of new treponema-based assays should be to correlate highly with resolved FTA-ABS/TP-PA results, as well as minimize the overall number of equivocal or inconclusive results.

All three assays had high accuracy (96.4 to 99.4%); however, they varied greatly in the number of equivocal results. The large number of equivocal results generated by both the Marblot and Virotech assays limits their utility. Table 2 illustrates that the ViraBlot assay performed the best when resolving inconclusive FTA-ABS results, not only by having good correlation with TP-PA but by having no equivocal results.

The Marblot assay is a traditional Western blot assay, while the ViraBlot and Virotech assays are immunoblot assays with purified antigens added to the strip. Advantages of the immunoblot assays over the Western blot assay were the consistency of the appearance of each band and the use of fewer bands for interpretation. This allowed easier interpretation and reduced the number of equivocal results.

Western blot and immunoblot assays offer additional, accurate treponemal tests that can supplement the current syphilis testing algorithm. As an esoteric reference laboratory, our laboratory often receives specimens for FTA-ABS testing that were previously inconclusive by FTA-ABS at another laboratory. Our data indicate that the ViraBlot assay would be the best choice of blot assay to use to resolve these inconclusive FTA-ABS results.

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